THE EFFECTS OF MICROWAVE RADIATION ON THE INFECTIVITY OF *TRICHINELLA SPIRALIS*

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The problem. The effects of microwave radiation on the infectivity of *Trichinella spiralis* in young, white, laboratory mice were studied.

Procedure. Mice were divided into two groups: 1) mice receiving isolated, irradiated larvae and 2) mice receiving larvae which were isolated from irradiated carcasses. These two groups were further subdivided by the time of microwave exposure of the larvae. Five weeks following inoculation, the diaphragms of mice were examined for the presence of *T. spiralis* larvae. The number of larvae present in the diaphragms were counted.

Findings. Isolated larvae exposed to microwave radiation for 12 seconds were rendered unable to reproduce in mice, and therefore non-infective. Isolated larvae exposed to radiation for 8 seconds produced a pronounced fewer number of larvae than did the unirradiated controls. Encysted larvae in mice carcasses which were irradiated for 18 seconds and then inoculated into mice were unable to reproduce and were non-infective. Encysted larvae irradiated for 14 seconds produced a pronounced fewer number of infective larvae than did the unirradiated controls.

Conclusions. The encysted larvae are slightly more resistant to microwave radiation than isolated larvae. Microwave radiation inhibits *T. spiralis* from maturing and producing larvae.

Recommendations. Further studies should be done using pork to determine if the results obtained in mice apply to other warm-blooded animals. A study to determine if irradiated larvae are unable to reach maturity or are rendered sterile when maturity is reached would be helpful in understanding the life cycle and reproductive capabilities of *T. spiralis*.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION AND REVIEW OF THE LITERATURE

The nematode Trichinella spiralis was discovered by James Paget in London, England in 1835. James Owen described the organism in a paper in 1835 and gave it the name Trichina spiralis. In 1896 the name was changed to Trichinella spiralis since Trichina had been used previously for a genus of Diptera and also for a genus of beetles (Gould 1970).

Some investigators recognize as many as three subspecies of T. spiralis. They are identified as T. spiralis spiralis, T. spiralis nativa and T. spiralis pseudospiralis. A scanning electron microscopy study shows no difference in surface morphology. The question of whether the epidemiology of trichinosis is complicated by the presence of more than one species has not been answered, and it is important that the nomenclature reflect this (Lichtenfels et al. 1983).

T. spiralis is a parasitic nematode found almost exclusively in warm-blooded carnivores. They are the cause of a condition known as trichinosis, which occurs as a result of the larval stage of T. spiralis invading the skeletal muscle of the host animal. An animal usually acquires the infection by eating meat of another animal which contains the larvae.

Trichinosis is widely found in Germany, Poland, Spain, Hungary and the lower Danube countries. Occasional epidemic outbreaks occur in parts of Latin America and the United States (Faust et al. 1975).

In 1979 there were 135 reported cases of trichinosis in the United States with only one being fatal. Since 1947 there has been a general decline in the number of cases of trichinosis which is partially due to a decrease in the number of garbage fed swine. In 1979, 29 cases of T. spiralis infections were reported in New Jersey, 27 in Alaska, and 22 in Louisiana. The Alaskan outbreak was due to people eating an infected walrus. The Louisiana outbreak was due to people eating homemade sausage from a garbage-fed pig, which was a violation of Louisiana law. Over the past five years, 1975-1979, Alaska, Iowa, New Jersey, Connecticut and Rhode Island have had the highest mean annual incidence rates. Iowa is in this group due to a 1975 outbreak which was the largest single source outbreak in history. Alaska's high incidence is probably due to the consumption of bear and walrus meat. Swine, walrus, and bears are the animals most commonly infected (Center for Disease Control 1979).

An outbreak of local interest occurred in December 1975 in which 73 people were diagnosed as having trichinosis in northeast Iowa. These patients were among 242 people who had eaten homemade produced pork/venison sausage. It was established by laboratory tests that the pork was infected with T. spiralis and the pork during cooking and smoking had reached a temperature of only 51.7^{o}C (Center for Disease Control 1976).

The life cycle of T. spiralis is unique for nematodes

in that all stages are within a warm-blooded animal. When a carnivore eats muscle of an infected animal, the larvae contained within that muscle are released as digestion of the muscle occurs. The larvae then invade the intestinal mucosa, usually in the duodenum (Faust et al. 1975). The larvae proceed through a series of four molts until sexual maturity is reached (Kozek 1971). Copulation takes place approximately 40 hours after ingestion has occurred (Gould 1970). By the 6th to 7th day after ingestion, live larvae are released by the female which reach the mesenteric venules (Gould 1970) or lymphatics and become distributed via the blood stream into the peripheral blood capillaries where they then enter into the skeletal muscle (Reece 1977). The female keeps releasing larvae for 4 to 16 weeks and may produce 500 larvae if the infection is light. In heavier infections, larvae output per female is less (Khamboonruang 1971). Each female may produce up to 1500 larvae in her lifetime (Jones 1967). The larvae begin encapsulation about the 21st day after birth by producing an ellipsoidal sheath. These capsules run in the same direction as the muscle fiber. Some larvae remain viable for many years within the capsule, while others become calcified between the sixth and twelth month (Faust et al. 1975).

The adult males are about 1.5 mm in length. Within a week after copulation they are passed out of the intestine. The adult female is 3-4 mm in length and may live for up to

2 to 3 months. The larvae are about 5-6 microns in length at birth, but when they reach the muscle tissue, they grow rapidly to a length of 250 to 500 microns. Cyst formation is complete in 7 to 8 weeks after the larvae have entered the blood stream (Jones 1967). A study of T. spiralis larvae suggests that larval length is dependent upon the host's and the parasite's physiology (Harley and Gallicchio 1971).

Trichinosis is an incurable and sometimes fatal disease. It is estimated that 30 larvae/gram of animal would be fatal to a rat, while only 5 larvae/gram of animal would be fatal to a human. The estimate for humans is based on larval counts in fatal cases, but since symptoms vary widely in humans, this count might not be totally accurate (Jones 1967). In mice, the weight of the animal does not directly correlate with the number of larvae found (Matz 1970).

There are three stages of the disease trichinosis. The first stage lasts 7 days (Faust et al. 1975). The symptoms are diarrhea, pain and nausea. The second stage lasts from about the 2nd week to the 6th week. This stage is due to the migration of larvae to the muscles. Muscles most heavily parasitized are the diaphragm, larynx, base of the tongue, abdomen, intercostals, biceps,psoas, pectorals, gastrocnemius, and the deltoids (Faust et al. 1975). The masseters, especially the left masseter, is also very highly parasitized (Olsen et al. 1964). Death often occurs during this stage. Inflammatory reactions are common and cause

severe pain. Breathing, chewing and vision may be affected and the eosinophil count may rise to over 50% during this stage (Jones 1967). Body temperature rises to 39° to 40°C and edema is also common, especially of the face (Faust et al. 1975). The third stage begins after the 6th week of the infection. If the infected person survives this stage, he will usually recover. Edema persists during this stage especially around the eyes, head, feet, and conjunctiva. Anemia, skin eruptions and pneumonia are symptoms of this stage (Jones 1967). Congestive heart failure may also occur (Faust et al. 1975).

There is no treatment of trichinosis although some of the symptoms are treatable. If the disease is detected early enough, the disease can be arrested before any pronounced symptoms occur. One of the first breakthroughs for the treatment of trichinosis came in the 1950's when physicians reported a symptomatic response to continuous massive doses of ACTH (adrenocorticotropic hormone). In patients receiving this treatment, there occurred a decline in fever, disappearance of aches and pains, and a resolution of rashes and edema. This treatment remains controversial since experimental animals treated with ACTH retain adult worms longer and more larvae are recovered from them. The animals also failed to develop an immunity to reinfection and there was also an increase in the mortality rate. On the other hand, infected muscles were only slightly inflammed and

encapsulation of the larvae was retarded. The use of ACTH is no longer considered as a safe treatment for humans as a result of experimental results with animals (Gould 1970).

Prednisone (a corticoid) has been used in the treatment of trichinosis. In laboratory mice, prednisone treated mice showed a 23% higher frequency of larvae present in the diaphragm muscle than did the non-prednisone infected control group (Reece 1977). Prednisone is an anti-inflammatory agent which is the basis of its use in the treatment of trichinosis. In a 1975 case report, good clinical response was reported by a man infected after eating bear meat who was given prednisone (1 mg/kg body weight per day). No toxic effects were observed, however, in comparison with pre-treatment biopsy, a more intense inflammatory response was seen following the subsequent administration of thiabendazole (Jordan et al. 1975).

Thiabendazole seems to be the drug of choice for trichinosis. It has been shown that thiabendazole is almost 100% effective against intestinal forms of T. spiralis when given in adequate doses (50-60 mg/kg body weight per day). It is most effective within 2 hours after ingestion of the larvae and its effectiveness decreases later in the day. Thiabendazole treated mice yielded only 10% of the number of larvae recovered from untreated controls (Gould 1970).

Thiabendazole has also been used to treat humans. In one study, over half of the patients manifested symptoms

of intolerance to the drug. In another study, 25 of 31 patients who had moderately severe trichinosis showed improvement, while none of six patients with long-standing trichinosis benefited from thiabendazole. There are side effects of thiabendazole treatment in humans which include aversion to the drug, aggravation of symptoms, dizziness, drowsiness, unpleasant dreams, tremors, fatigue, liver neurosis, vomiting, diarrhea, abdominal pain, fever, and anaphylactic shock (Gould 1970). If thiabendazole is combined with a corticosteroid in order to combine the antihelminthic effect of one drug with the anti-inflammatory effect of the other, larvae are suppressed as long as thiabendazole treatment is continued. It would be of importance to clarify whether such drugs as hydrocortisone reduce the effect of the antihelminthic (Campbell and Blair 1974). Other studies have shown that cortisone itself causes decreased enteritis in mice, but an increase in the fecundity of the adult worms (Stewart et al. 1982).

A further study in the control of trichinosis has been the use of ethanol. In adult rats administered 4 ml of 30% ethanol after T. spiralis inoculations, the rats were shown to be fully protected. It has been suggested that pigs be given Irish whiskey after meals. This treatment depends more on the promptness of treatment than on body weight dosage (Campbell and Blair 1974).

The use of chemotherapy is controversial because of

the side effects of the drugs. Cortisone might help reduce muscle swelling but increase the fecundity of the worms. While thiabendazole has some advantages, its side effects can be severe. This is why it would not be wise to administer it to animals which later would be used for human consumption.

It is widely accepted that the most effective ways of preparing infected meat for humans is by adequate freezing and cooking. The thermal death point of T. spiralis is 55°C. It is suggested that all meat products suspected of hosting T. spiralis be cooked at 137°C and to be certain that all parts of the meat reach that temperature (Gould 1970).

The World Health Organization recommends that pork be cooked at 30 to 35 minutes/kg and larger cuts for twice as long. Trichinella larvae can also be destroyed by freezing. The regulations of the Consumer Marketing Service require that cuts of pork not over 6 inches (15 cm) thick be exposed to -15°C for 20 days, to -23.3°C for 10 days, or to -28.9°C for 6 days. A temperature of -37.2°C for 2 minutes at the center of trichinous meat will kill the larvae (Gould 1970).

The arctic form of Trichinella has been designated as T. nativa (Margolis et al. 1979). The reason for this specific identification is the larval tolerance of cold temperatures. Viable larvae have been found in the meat of arctic species that have been frozen for several weeks at

-32°C and in another case in which meat had been frozen for one month at -12°C. This is usually believed to be the arctic species that is more tolerant to cold temperatures (Margolis et al. 1979).

The use of radiation of both larval and adult forms has also been used in an attempt to control trichinosis. If Trichinella spiralis larvae are exposed to x-rays (an ionizing form of radiation) greater than 1200 R, the larvae are able to develop into adults and the number of offspring is greatly reduced. It was also found that with an exposure of 4000 R, a few larvae could still infect the muscle of rats. An exposure of 5000 R or more not only reduced the number of larvae that would infect muscle, but also reduced the number of larvae able to reach adulthood. Some levels of radiation allow the development of intestinal infection but prevent subsequent muscle invasion. It was further observed that intestinal infections alone could produce at least a temporary immunity to further infections with non-irradiated trichinae. Trichinae exposed to 12,000 R of cobalt-60 gamma radiation (also an ionizing form of radiation), prevent the larvae from reproducing and when larvae are exposed to 10,000 R or more and then fed to rats, the larvae are incapable of reproducing. Increasing the exposure to 30,000 R and greater results in a failure of the larvae to reach maturity, and if exposed to 100,000 R and greater, the larvae would not remain in the intestinal tract of the host for more than 24 hours (Gould 1970).

Gould confirmed some of the previous results when he found that exposure of adult worms to 10,000 R of cobalt-60 gamma radiation rendered most of the worms sterile. Larvae exposed to 18,000 R did not reach maturity. Irradiation at 10,000 R permitted larvae to reach maturity and the host would develop a definite degree of immunity to reinfection by non-irradiated larvae. If the radiation was 18,000 R, little or no immunity resulted (Gould 1970).

The most obvious effect of ionizing radiation is related to cell division. The length of mitotic inhibition increases with the amount of radiation given to the cells. Consequently it would be expected that the larvae would be affected more than the adults (Gould 1970).

It is estimated that meat would need to be exposed to radiation as high as 100,000 rads to kill the larvae if present. This would probably alter the protein and enzymes found in the meat and have an effect on the taste of the meat. Irradiation and cooking of meat together could be of an advantage because following a high dose of radiation, no larvae would be left undamaged. This could help to prevent an onset of trichinosis even if portions of the meat were inadequately cooked (Gould 1970).

The use of microwave radiation as a control measure for T. spiralis has not been reported. Microwaves are a form of non-ionizing electromagnetic radiation in the range of 30-3000 mHz (Leonard et al. 1983). This corresponds to a

wavelength of 1 meter to 1 millimeter in air. The United States of America Standards Institute considers the frequencies of 10 and 100,000 mHz as the boundary of the microwave region (Baranski and Przemyslaw 1976). Microwave ovens commonly operate at 2450 mHz which is one of the bands reserved for industrial, scientific, and medical purposes (Leonard et al. 1983).

In biological systems, absorbed microwave energy is transformed into increased kinetic energy of the absorbing molecules, thereby producing a general heating of the tissues (Michaelson 1974). The molecules that make up food (muscle) when placed in a microwave oven are rotated 180^{o} from their starting position and back 2450 million times a second (2450 mHz). This constant rotation of the molecules causes the food to heat. As the wave penetrates the food, the power becomes less intense and decreases with each successive layer of molecules. The interior of the food is heated by conduction from the heat generated at the surface of the food (Wirtz 1984).

The depth of penetration of microwaves into tissues is dependent upon water contact and is about 5 times greater for tissues of low water content than those of high water content (Schwan 1969). At a frequency of 1 gHz, the wave penetration is limited to 1.5 to 2 cm in tissues with high water content but is extended to 6 to 8 cm in tissues with low water content. At microwave frequencies, the macroscopic

dielectric properties of tissues are strongly determined by their water content and thus are similar to those of pure liquid water (Leonard et al. 1983).

Tissues such as brain, muscle, and skin have high water content and have higher permittivity (Leonard et al. 1983). The lens of the eye is most sensitive because of its poor ability to lose heat and its closeness to the surface of the animal. The second most sensitive organ is the testis for the same reasons as the lens of the eye, plus its high sensitivity to heat. The body of an animal as a whole would rank third since it can tolerate only a limited increase in temperature and has only a limited ability to lose heat (Ely et al. 1964).

Heat is the major reason that microwaves may alter an organism or structure. Most results on mutagenicity of microwaves are negative or can be explained by temperature enhancement. The rapid heat production associated with microwaves do not apparently result in detectable alteration of cell cytoplasm and biomembranes (Leonard et al. 1983).

In prokaryote cells, RNA and DNA have been shown to absorb microwave radiation and alter the metabolic processes and growth of Escherichia coli. In eukaryotic cells, negative results have been reported with the use of microwave radiation. There is some evidence of a possible synergistic effect between microwaves and recognized mutagens. In Drosophila no demonstrable evidence for mutagenic effects have

been shown. However, female flies exposed to 2450 mHz radiation produced fewer, less viable eggs than did the controls. Microwave exposure of rabbits and rats and mice have given both positive and negative results on the growth of offspring. In human lymphocytes exposed to microwave radiation, lymphoblastoid transformation has been observed, and since under the laboratory conditions used there was no rise in temperature, the results are not attributed to heat (Leonard et al. 1983).

In a study done with Nippostrongylus brasiliensis, larvae exposed to 9 cm microwave action for 30 seconds developed in the same manner as untreated controls, but larvae exposed to microwave action for 180 seconds stopped the development of N. brasiliensis females (Duk et al. 1979).

In a study with Strongyloides ratti exposed to microwave radiation, microwaved infective larvae of S. ratti were immunogenic for rats, even though they were incapable of reaching the infective stage and maturing to adult worms (Condor and Williams 1983).

The purpose of this study was to determine if microwave radiation influences the number and distribution of T. spiralis in the skeletal muscles of mice. The study will contribute information relating to the control of T. spiralis and the biological effect of microwave radiation. The study will further serve to identify the feasibility of using microwave radiation for the cooking of foods having T. spiralis.

MATERIALS AND METHODS

Young adult male albino mice, used in this study, were obtained from SASCO, Omaha, Nebraska. At the time of inoculation, each mouse weighed approximately 20 grams. The mice were given water and Purina brand lab chow ad libitum throughout the study; however food was withdrawn four hours pre-inoculation, and for four hours post-inoculation. This was done to increase the chances of infectivity of the mice to T. spiralis. All mice were housed in appropriately labeled plastic cages which were lined with sawdust and no more than five mice to a cage. The Trichinella spiralis larvae used in this study were obtained from a strain maintained by the Biology Department at Drake University.

The mice used in this study were divided into two groups. In Group I the mice were inoculated with infective T. spiralis larvae that had been isolated and exposed to microwave radiation. This group was further divided into nine subgroups, each containing ten mice. In each subgroup, the infective isolated larvae were irradiated for varying time intervals of 2, 4, 6, 8, 10, 12 and 14 seconds. Two control groups were also established with one group receiving non-irradiated larvae and the second group designated as the normal, uninfected control (see Table 1).

In Group II the mice were inoculated with infective T. spiralis larvae that had been recovered from infected

mouse carcasses treated with microwave radiation. This group was further divided into eight subgroups, containing ten mice each. In each subgroup, the mouse carcasses containing infective larvae were irradiated for varying time intervals of 5, 10, 12, 14, 16 and 18 seconds. Again, two control groups were established, one group receiving non-irradiated larvae and the other group serving as the normal uninfected control (see Table 2).

Table 1. Schedule of Inoculations. Group I. Mice Infected with Isolated T. spiralis Larvae Following Treatment with Microwave Radiation.

Subgroup	Number of Mice in Each Group	Number of Larvae Given to Each Mouse	Treatment Time of Larvae
Control	6	none	none
Infected Control	6	120	none
Experimental A	10	120	2 sec.
Experimental B	10	120	4 sec.
Experimental C	10	120	6 sec.
Experimental D	10	120	8 sec.
Experimental E	10	120	10 sec.
Experimental F	10	120	12 sec.
Experimental G	10	120	14 sec.

All exposure to radiation was done in an Amana Microwave oven (Amana Refrigeration, Inc., Amana, Iowa), Model

RRL-8TD, at 100% power (approximate power output, 700 watts, 2450 mHz) available in the Biology Department at Drake University.

Table 2. Schedule of Inoculations. Group II. Mice Infected with T. spiralis Larvae Recovered from Infected Mouse Carcasses Following Treatment with Microwave Radiation.

Subgroup	Number of Mice in Each Group	Number of Larvae Given to Each Mouse	Treatment Time of Carcass
Control	10	none	none
Infected Control	10	120	none
Experimental A	10	250	5 sec.
Experimental B	10	80	10 sec.
Experimental C	10	110	12 sec.
Experimental D	10	110	14 sec.
Experimental E	10	130	16 sec.
Experimental F	10	100	18 sec.

To check heat levels, it was necessary to see how much irradiation was needed to raise 5 ml of room temperature water to 55°C, the lethal heatpoint of T. spiralis. Room temperature water (22°C) was used since the solutions containing the infective larvae were at room temperature before being irradiated. The heat levels were checked by using the temperature probe that was included with the oven. The probe was placed in a 10 ml pyrex beaker containing 5 ml of

distilled water at room temperature. The oven was programmed to heat the water to 87.8°C. The power was activated and when the temperature reached 54.4°C the power was shut off. A Heur stopwatch was used to determine the time needed to reach the desired temperature. The probe was left in the beaker to determine how high the temperature would continue to rise, and the time needed for the temperature of the water to cool back down to 54.5°C. The same procedure was used in heating the water to 43.3°C, 48.9°C and 60.0°C.

Using the temperature probe, after 10 seconds of exposure, the temperature of 5 ml of distilled water at room temperature (23.9°C) increased to 43.3°C. After 12 seconds, the temperature increased to 48.9°C; after 14 seconds, it remained at 48.9°C; after 16 seconds, it was 54.4°C; and after 18 seconds the temperature was 60.0°C (see Table 3).

The time intervals of exposure to radiation were determined by three pilot studies. The first study was designed to determine the amount of microwave radiation that isolated larvae could withstand before death occurred as measured by loss of movement. The second study was done to determine the range of infectivity of T. spiralis by exposing isolated larvae to radiation and subsequently inoculating mice. The third pilot study used mice carcasses infected with T. spiralis, irradiating them for different time intervals and then isolating the larvae and inoculating mice. This later study was done to determine the range of infec-

tivity of larvae subjected to this treatment.

Table 3. Temperature of 5 ml of Distilled Water After Exposure to Microwave Radiation at Different Time Intervals.

Time in Seconds	Temperature in C degrees	Maximum Temperature in C degrees After Power was Discontinued	Time in Minutes Required for Temperature to Return to 54.4°C
0	23.9	-	-
10	43.3	48.9	-
12	48.9	54.4	-
14	48.9	65.6	1:30
16	54.4	65.6	2:07
18	60.0	65.6	2:10

The infective T. spiralis larvae were removed from the stock mice by sacrificing the mice using cervical dislocation. The mice were skinned, and the feet, tail, and head removed. To establish that the mice were infected, the diaphragm was removed by making a midsaggital incision through the ventral surface of the mouse and carefully detaching the diaphragm from the body wall by the use of iridectomy scissors. The diaphragm was examined with the low power objective of an American Optical compound microscope, utilizing the diaphragm press method. After the presence of the larvae in the mice were confirmed, the mice were eviscerated leaving only the skeleton and skeletal muscles. The carcass was

rinsed in 0.85% saline solution to remove blood and any attached debris.

The remaining carcass was cut into approximately 2 centimeter segments and placed in a Waring Blender (Waring Products Division, Dynamics Corporation of America, Hartford, CT) with 500 ml of pepsin solution (500 ml distilled water, 10 ml concentrated hydrochloric acid, 2.5 grams pepsin (Fischer Scientific). The carcass pieces and pepsin solution were blended for 40 seconds at high speed at approximately 17,000 rpm (Gallogly 1968). This mixture was then poured into 250 ml screw top Erlenmeyer flasks. The blender, after using, was rinsed with 50 ml of pepsin solution which was then added to the flasks. The flasks were then placed in an Eberbach Waterbath Shaker (Eberback Company, Ann Arbor, MI) for 4 hours at 37°C to allow the larvae to become digested from the muscle tissue.

After 4 hours had elapsed, the digest was sampled for T. spiralis larvae to establish that the larvae had been released from the host tissue. This was done by examining a drop from the digest solution microscopically with the low power objective, to check for isolated larvae. Then the solution in the flasks was allowed to settle for 20 minutes. After settling, 200 ml of solution was aspirated from the top of each flask, and 200 ml of warm water (37°C) was added. The washing was done three times at 20 minute intervals. After the final washing, the supernate of each flask was

aspirated off, and the remaining solution in each flask was combined into one flask. The flask was shaken periodically to ensure even distribution of the larvae throughout the solution.

The larvae were removed from the solution by using a 9 inch Pasteur pipette. Five drops of the solution were examined microscopically to determine the number of larvae in 1 drop. In Group I, an average of 4 larvae per drop was found. The calibrated pipette used for inoculations had 30 drops per ml, therefore 1 ml of solution contained 120 larvae.

The solution containing the isolated larvae was distributed into sixteen 10 ml pyrex beakers, with 5 ml of solution in each beaker. Each beaker was placed individually in the microwave oven. Two beakers containing T. spiralis larvae were irradiated for each time period listed previously (Table 1). Two beakers were not irradiated and were used as the control.

The preparation for the second group was done slightly differently. The mice were killed, skinned and eviscerated in the same manner as described. In this part of the study, seven different carcasses were used. Each carcass was placed on an inverted petri dish, covered with a weighing paper and irradiated for time periods of 5, 10, 12, 14, 16 and 18 seconds respectively (Table 2). One carcass was not irradiated and was used as the control. The carcasses were then individually placed into the Waring blender with the pepsin

solution, and blended as described previously. The mixture was then placed into individual flasks. The blender, after using, was washed with tap water between blendings. The flasks for each group were put into the shaker waterbath as previously described for 4 hours, and then the contents were allowed to settle. The larvae were washed in the same manner as previously described. It was not necessary to transfer the larvae to 10 ml flasks since irradiation had already taken place.

Using the same counting method as described earlier, the number of larvae given to each mouse is stated in Table 1. One ml of solution (30 drops) containing the infective larvae was given to each mouse. In both parts of the study, solutions were checked to be certain that the larvae were still alive after irradiation.

To prepare the mice for inoculation, each mouse was lightly anesthetized in a glass jar with diethyl ether (Mallinckrodt, Inc.). A 1 ml calibrated pipette was filled with the appropriate solution to the 1 ml mark. The pipette was previously fire polished to ensure that the mouse would not be cut during intubation. The anesthetized mouse was held by the skin on the back of the neck and then pulling the head of the mouse slightly posteriorally. The pipette containing the solution was gently eased down the esophagus of the mouse and the 1 ml of solution containing the Trichinella larvae were released into the stomach of the mouse.

Following recovery from the anesthetic, the mouse was then placed in the appropriately labeled cage.

After 35 days, the experimental mice were sacrificed by cervical dislocation. The mouse was checked for infection by removing the diaphragm in the same manner as previously described. A diaphragm press was then prepared and examined microscopically and the number of larvae of T. spiralis present in the diaphragm were counted.

DATA AND DISCUSSION

Following the inoculation of the mice used in this study with Trichinella spiralis, the mice were observed daily and no adverse effects were noted other than an occasional scraggly appearance. All mice showed a normal weight gain and no unusual changes in development were noted.

Several mortalities were noted during the study. In Group I, in which isolated larvae were treated with microwave radiation, 1 mouse of the 10 died in the 2 second microwave exposure group, and 1 mouse died in the 6 second and in the 8 second exposure groups. In Group II, in which the infected carcass of mice was irradiated and then the larvae isolated and inoculated into mice, there were 4 deaths in the 10 second group out of the 10 mice inoculated. However, these deaths were due to the aggressiveness of 1 mouse in a cage of 5 who killed the other 4 mice in the cage. No mortalities occurred in any other group.

In Group I in which mice were inoculated with isolated T. spiralis larvae following exposure to microwave radiation, the larval counts are shown in Table 4. All mice were inoculated with 120 infective larvae which had been exposed to microwave radiation. Diaphragm counts showed that a reduction in the number of larvae occurred as the time of exposure to microwave radiation was increased. The unirradiated control mice showed a mean larval count of 288.3; the 2 second exposure was 261.2; 4 second exposure was 232.2; 6 second exposure was 229.6; 8 second exposure was 151.0; and 10 second exposure was 69.1. The larvae which were exposed to microwave radiation for 12 seconds and for 14 seconds showed no larvae present in the diaphragms of the mice.

The data shown in Table 4 is illustrated in graphic form in Figure 1. The graph shows a fairly constant decline in number from the unirradiated control group through the 6 second microwave irradiation group. The number of larvae present in the diaphragms of mice is greatly reduced as the exposure time of radiation is increased beyond 6 seconds, so that no larvae could be demonstrated in the mice diaphragms at either the 12 second or 14 seconds of exposure. This would suggest that the exposure of isolated T. spiralis larvae at 2450 mHz for 12 seconds either rendered the larvae sterile or prevented them from reaching maturity. The 8 second microwave exposure time is a critical time period, since the number of larvae produced declines abruptly at this time

Table 4. Frequency of T. spiralis Larvae in Diaphragms of Group I Mice Receiving Isolated Irradiated Larvae.

Control	2 sec	4 sec	6 sec	8 sec	10 sec	12 sec	14 sec
370	174	0	379	131	171	0	0
310	101	229	466	76	0	0	0
271	381	319	109	201	0	0	0
349	155	277	0	109	0	0	0
231	299	245	199	85	41	0	0
199	233	229	164	103	89	0	0
	364	222	375	190	0	0	0
	298	238	129	249	140	0	0
	346	304	246	225	420	0	0
	-	260	-	-	0	-	-
Mean 288.3	261.2	232.3	229.7	151.0	69.1	0	0
PerCent Reduction in Larvae -	9.3	19.4	20.3	47.6	76.0	100.0	100.0

- = animals died during the study

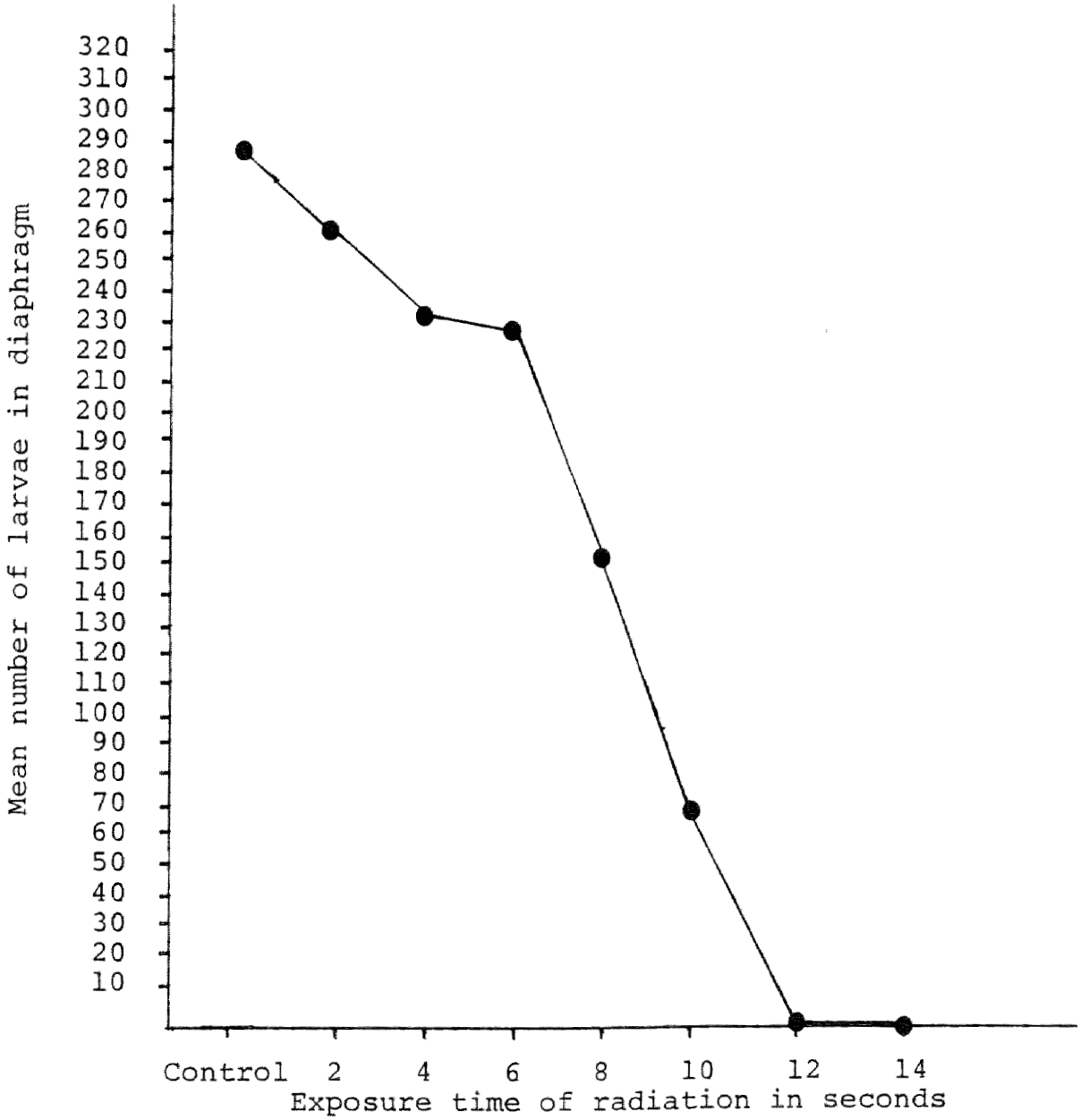

Figure 1. Mean number of T. spiralis larvae recovered from diaphragms of mice inoculated with isolated larvae exposed to microwave radiation.

at this time of exposure. At 8 seconds, a 47.6% decrease in the number of larvae was found as compared to the control.

In Group II the carcasses of mice infected with T. spiralis were exposed to microwave radiation for varying time periods, the larvae isolated and subsequently inoculated into mice. In this study, the mice in each group were inoculated with varying numbers of T. spiralis larvae, the number depending on the total number of larvae recovered from the irradiated carcass as shown in Table 2.

The T. spiralis larvae recovered from the mouse diaphragms are presented in Table 5 and the calculated mean for each group is listed. However, in order to better compare the effects of microwave irradiation, an adjusted mean was determined by converting the mean values obtained to a standard 120 larvae inoculated. The unirradiated control shows an adjusted mean of 153.6 larvae per mouse diaphragm. The mouse carcass exposed to 5 seconds of microwave irradiation shows an adjusted mean of 99.2 larvae per diaphragm; 10 seconds exposure 103.7 larvae; 12 seconds exposure 68.5 larvae; 14 seconds exposure 82.2 larvae; and 16 seconds exposure 26.2 larvae. No larvae were recovered from the diaphragms of any mice in the 18 second exposure group. These results suggest that larvae in a mouse carcass for 18 seconds at 2450 mHz are sterilized by the procedure or are unable to reach maturity.

The data shown in Table 5 is illustrated in graphic

Table 5. Frequency of T. spiralis Larvae in Diaphragms of Group II Mice Receiving Isolated Larvae from Irradiated Infected Mice Carcasses

	Control	5 sec	10 sec	12 sec	14 sec	16 sec	18 sec
	179	191	40	40	71	49	0
	200	166	53	77	85	31	0
	114	131	81	81	104	27	0
	171	274	130	84	110	12	0
	116	194	39	32	90	41	0
	183	162	81	36	61	30	0
	164	171	-	60	49	34	0
	91	183	-	91	67	22	0
	66	307	-	78	40	36	0
	252	288	-	49	-	-	0
Mean	153.6	206.7	69.2	62.8	75.3	28.4	0
Adjusted Mean	153.6	99.2	103.8	68.5	82.2	26.2	0
PerCent Reduction In Larvae	-	35.4	32.4	55.4	46.1	82.9	100.0

- = animals died during the study

form in Figure 2. The decline in the average number of larvae recovered from the mice diaphragms for the microwave exposure time is clearly shown. The sharpest decline in larval counts occurs after 14 seconds of exposure to microwave radiation suggesting that 16 seconds is a critical time period for T. spiralis larvae. At 16 seconds, a 82.9% decrease in the number of larvae was found, as compared to the controls.

The results of this study seem to indicate that in a comparison of Group I and Group II studies, the carcass of the mouse either protects the larvae from irradiation or absorbs some of the microwaves. By comparing the 10 second irradiation of isolated larvae in Group I with the 10 second irradiation of the Group II T. spiralis infected mouse carcass, the larval diaphragm count increased from 69.1 to 103.7. In irradiation for 12 seconds, no larvae were recovered from the diaphragms in Group I whereas 68.1 larvae were recovered from Group II. No larvae were recovered from the Group I larvae irradiated for 14 seconds whereas 82.1 larvae were recovered from Group II mice inoculated with larvae irradiated within the mouse carcass. It required 18 seconds of exposure of the mouse carcass to microwave radiation to render Group II T. spiralis larvae non-infective, whereas it required only 12 seconds of exposure of the Group I isolated larvae to produce the same results. Tissues which are high in water content have about the same dielectric property as pure water (Leonard et al. 1983) which supports the idea

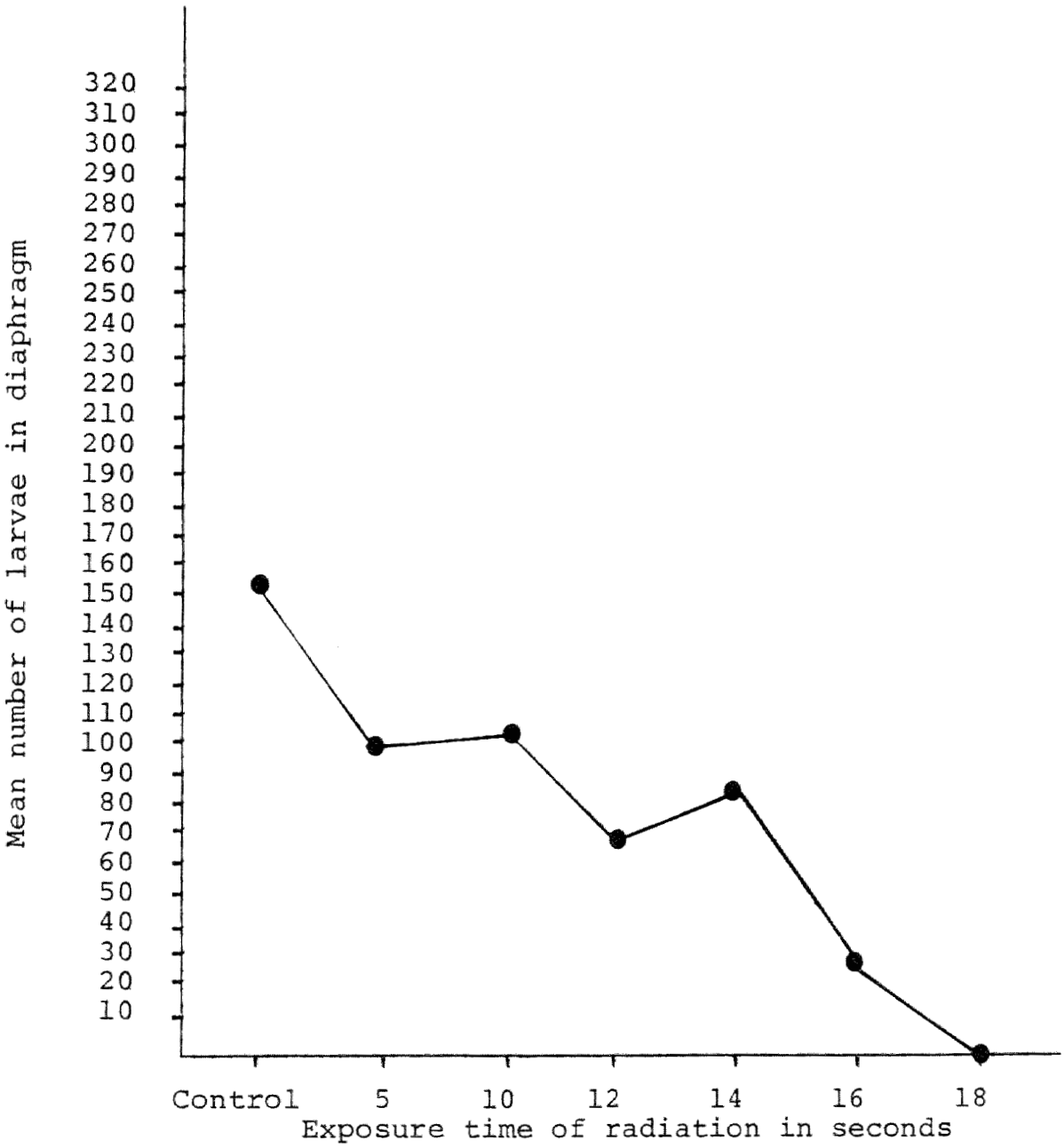

Figure 2. Mean number of T. spiralis larvae recovered from diaphragms of mice inoculated with larvae isolated from irradiated infective carcasses.

that the carcass infected with T. spiralis larvae absorbs some of the microwave radiation, rather than actually offering protection.

Duk et al. (1979) studied the effect of microwave radiation on Nippostrongylus brasiliensis, an egg laying nematode, by determining the number of eggs produced following varying exposure times. Using the rat as the host, he showed that N. brasiliensis larvae irradiated for 180 and 210 seconds produced no eggs following inoculation into the rat, whereas this study with Trichinella spiralis showed that no larvae were produced following exposure to microwave radiation for 12 seconds. The power (mHz) of the microwave source used by Duk was not stated which could explain the difference in results, or perhaps N. brasiliensis contains properties which render them more resistant to microwave radiation.

A study done on the infective larvae of Strongyloides ratti (Condor and Williams 1983) showed that larvae irradiated for 15 seconds at 100% power of a Tappan microwave oven were unable to reach maturity. Their study used a 15 second exposure time since it was designed to show immunologic effects. The results of the 15 second exposure time required to inhibit the maturity of S. ratti would correspond to the 12 second exposure time observed in this study with T. spiralis. The 3 second difference in exposure time might be a function of the brand of microwave oven used, or a difference in the ability of the helminths to withstand microwave radiation.

This study with Trichinella spiralis cannot decisively explain if the inhibition of maturity, or failure to reproduce, was due to heat production or radiation. A study of heat production in 5 ml distilled water (Table 3) in 10 ml pyrex beakers showed that the lethal death point for T. spiralis (55°C) was reached in 16 seconds. At this time of exposure, the temperature continued to rise to 65.5°C following the discontinuance of the irradiation. Approximately two minutes were required for the temperature to return to 55°C again. The water irradiated for 12 and 14 seconds eventually reached the thermal death point after the power was shut off to the microwave oven (see Table 3). It was not possible to check the temperature of the irradiated carcass with the equipment available for this study. All irradiated larvae were alive (as determined by movement) and active when inoculated into the experimental mice in this study. It is not known if isolated larvae exposed to 55°C for the two minutes and twenty-three seconds will eventually die due to the heat exposure.

The data obtained from this study indicate that microwave radiation has an inhibitory effect on the maturation or reproduction of T. spiralis or a lethal effect depending on the time of exposure. It would be of interest to study the immunity or resistance of the host animal to a challenge inoculation of T. spiralis following previous inoculation of T. spiralis irradiated for 12 or 14 seconds. A further study

of interest would be to recover the helminths from the duodenum of mice and establish the degree of maturity achieved, following the inoculation of the mice with larvae irradiated for 12 and 14 seconds. If adult females could be obtained, it could then be assumed that the microwave radiation had induced sterility, either in the male, the female, or both. An additional study could be of interest by irradiating the larvae for longer periods of time at lower power settings, so that less heating would occur. This study could establish if the larvae are affected by heat, radiation, or a combination of both. With facilities available, it would be of some interest to repeat the experiments done in this study with different host animals such as rabbits and pigs. This would be of interest to establish if the host tissue protects the larvae from the effects of microwave radiation, and further if changes in the quality of skeletal muscle used for human consumption is changed by microwave action. The application of such procedures in controlling trichinosis in human populations would be of great interest.

CONCLUSION

The infectivity of T. spiralis larvae of the mice used in this study was determined by examining the diaphragm of the mice. Isolated larvae of T. spiralis, when exposed to microwave radiation, were rendered unable to reproduce at the exposure time of 12 seconds. Exposure time of 8 seconds

produced a pronounced fewer number of larvae than the unirradiated controls, or larvae irradiated for less time. Larvae still encysted in muscle, when exposed to microwave radiation at the 18 second exposure time were rendered unable to reproduce. Exposure of 14 seconds produced a pronounced fewer number of larvae than did the unirradiated control or larvae exposed to radiation for less time.

Larvae irradiated in the carcass appear to be protected by the carcass in some manner. It remains to be determined if this is due to the fact that microwaves do not penetrate through the muscle or if microwaves are absorbed by the carcass. It also was not determined if irradiated larvae were unable to reach maturity or if they reached maturity but were sterile.

It was determined that exposure to microwave radiation does have an effect on the life cycle of T. spiralis. This evidence could prove very beneficial in the ultimate elimination of future T. spiralis infections in humans.

Further studies of this problem are suggested by the use of microwave radiation upon infected pork meat, since this is a major source of T. spiralis infections for humans. Also studying the effect of radiation upon T. spiralis, to determine if larvae were rendered sterile or unable to reach maturity, would enhance the knowledge of the life cycle of the parasite and its reproductive capabilities.

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